

limitations relating to whole cell microorganisms, a low salinity concentration in a fermentation medium in which the microorganisms are cultured, and the content of omega-3 highly unsaturated fatty acids of the microorganisms as a percentage of total cell dry weight.

In the amendments to the Specification, on page 16, the change of "10%" to "15%" is to correct a typographical error; the correction is supported by the Declaration of William R. Barclay, paragraph 9, attached hereto and incorporated in its entirety herein by reference. The correction on page 53 of "Oomycetes" to "Thraustochytrids" is supported in the Specification on page 27, line 35 to page 28, line 30, by the taxonomic discussion that the species Thraustochytrium and Schizochytrium were reclassified from the order Oomycetes to the order Thraustochytriales. The insertion on page 53 of " KH_2PO_4 , 1 g/l" was to correct the recitation of the nutrients in the culture medium. The nutrient KH_2PO_4 is present in the culture medium set forth in other examples of the Specification, namely Example 4 (page 37, line 24), Example 6 (page 49, line 14), Example 9 (page 54, line 19), and Example 11 (page 57, line 6). The correction on page 54 of "39.0%" to "29.0%" and the correction on page 55 of "25.6%" to "15.6%" are corrections of typographical errors which are supported by the Declaration of William R. Barclay, paragraph 9. The attached Figure 6 is being substituted for Figure 6 as originally filed with the application, because in the originally filed Figure 6 the bars representing total fatty acids and omega-3 highly unsaturated fatty acids were misplotted for ATCC Accession No. 20889. These particular bars were misplotted because a graphics plotting computer program was used to generate originally filed Figure 6 and erroneous data was entered into the program (See Declaration of William R. Barclay, paragraph 10).

Rejection of Claims 1-8 and 10 Under 35 U.S.C. §112

Claims 1-8 and 10 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Specifically, the Examiner states that in Claim 1, the recitation of "microorganisms or extracted omega-3 HUFAs" is vague and indefinite. Applicant notes that in amended Claim 1 the suggested language of the Examiner has been adopted.

The Examiner also states that the recitation of "HUFAs" in the claims is vague and indefinite and that abbreviations should be defined in the first instance of use. Applicant notes that the abbreviation "HUFAs" has been eliminated in the claims as amended and "highly unsaturated fatty acids" has been substituted therefor.

The Examiner further states that the taxonomic order Thraustochytriales should be underlined or italicized. Applicant respectfully traverses the Examiner's position that the order Thraustochytriales should be underlined or italicized. Applicant respectfully submits that only the designation for genus and species should be underlined or italicized. As support for Applicant's position, Applicant refers the Examiner to Biology, Clyde F. Herreid II, pages 68-69, MacMillan Publishing Co., Inc., New York, New York (1977). A copy of these pages is attached for the Examiner's convenience.

The Examiner states that Claim 10 is vague and indefinite in its recitation and that the claim is confusing as its dependency appears to be incorrect. Applicant has amended Claim 10 to indicate that it is a process claim properly dependent upon Claim 9. Accordingly, Applicant respectfully requests that Claim 10 be included in the invention set forth in Group II of the restriction requirement and that Claim 10, as amended, be withdrawn from consideration.

Rejection of Claims 1-3, 8 and 10 Under 35 U.S.C. §102(a)

Claims 1-3, 8 and 10 are rejected under 35 U.S.C. §102(a) as being anticipated by Long. Applicant notes that Claim 10 has been withdrawn from consideration. The Examiner takes the position that Long discloses the production of omega-3 fatty acids by heterotrophic microorganisms from the genera Thraustochytrium and Schizochytrium and that Long also discloses the omega-3 fatty acids to be useful in nutrition as a nutritional additive to human diets, as well as, an animal feed additive. Applicant respectfully traverses the Examiner's rejection. Applicant respectfully submits that Long teaches the use of extracted omega-3 fatty acids (See page 6, lines 4-6). In contrast, the present invention as now claimed in amended Claims 1-8 claims the use of a whole cell microorganism containing omega-3 highly unsaturated fatty acids (HUFAs) (See page 20, lines 5-10 and amended Claims 1-8).

To demonstrate the advantage of the use of a whole cell microorganism with a high content of omega-3 highly unsaturated fatty acids as claimed by the present invention (Claims 1-8, 56 and 62), the following example is helpful. A laying hen consumes about 100 grams of feed per day. One of the strains taught by the present invention (ATCC Accession Number 20889) produces, at a minimum, 6.7 percent of its total cell dry weight as omega-3 highly unsaturated fatty acids. Thus, using the microorganism strains of the present invention, a laying hen would need to consume only about 6 grams of whole cell microorganisms per day to produce an omega-3 highly unsaturated fatty acid enriched egg employing the method of Example 14. (See Declaration of William R. Barclay, paragraph 14 and Example 14). In contrast, using the highest published omega-3 highly unsaturated fatty acid content for the strains taught by Long, one would have to feed about 42, 26, or 48 grams of dry cells per day of Nitzschia, Crypthecodinium, or Pythium, respectively, to a laying hen to achieve an egg with a

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claimed

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in scope
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one strain

omega-3 highly unsaturated fatty acid content employing the method of Example 14. Since laying hens only eat 100 grams per hen per day, this would require a laying hen to eat an equivalent of about 26 percent to 48 percent of its diet of the microorganism strains taught by Long in order to achieve the same effect. Replacing this amount of the hen's diet with whole cells of the strains taught by Long presents several problems: 1) it would most likely lead to nutritional imbalance in the hens and cause a cessation or serious reduction of egg production, because so much of the laying hen's normal diet will be substituted by the microbial cells; and 2) it would cost five to ten times as much to achieve the same effect using the strains taught by Long as achieved by feeding a laying hen the microorganism strains taught and claimed by the present invention (See Declaration of William R. Barclay, paragraph 14).

Claims are broader
Applicant respectfully submits that the present invention is a food product which has a source of omega-3 highly unsaturated fatty acids derived from microorganisms of the order Thraustochytriales which have one or both of the following economically desirable characteristics for the production of omega-3 highly unsaturated fatty acids:

- not claimed in all cases
scope problem
- 1) preferably euryhaline (able to grow over a wide range of salinities, but especially at low salinities) (See page 11, lines 3-16); and
 - 3) high content of omega-3 highly unsaturated fatty acids (measured as a percentage of total cell dry weight) (See page 11, lines 3-16; page 16, lines 4-8 and Example 9, page 54).

The presence of these characteristics in the microorganism strains taught and claimed by the present invention is advantageous. Specifically, the ability of these microorganisms to be cultured in a low salinity medium is economically advantageous, because the corrosive effects of saline waters on metal fermentors

and the problems associated with the disposal of saline waters can be avoided or mitigated (See page 54, lines 8-12). The high content of omega-3 highly unsaturated fatty acids of these microorganisms presents a further advantage over prior art strains. For instance, the present invention teaches and claims (Claims 56 and 62) that the omega-3 HUFA content of the microorganism strains (expressed as a percentage of total cell dry weight) of the present invention are greater than about 6.7 percent of total cell dry weight, which is greater than that of the prior art strains taught by Long (See Figure 6; Prior art strains are ATCC Accession Numbers 28211, 34304, 28210, 24473, and 28209; See also Declaration of William R. Barclay, paragraph 13).

Long teaches the production of omega-3 fatty acids by culturing heterotrophic microorganisms from the genera Thraustochytrium and Schizochytrium in a halo-tolerant or a halophilic medium, that is, a natural or artificial seawater medium (See page 4, line 32 to page 5, line 3 and page 5, lines 27-32). In contrast, the present invention teaches and claims the production of omega-3 HUFAs by culturing Thraustochytrium and Schizochytrium in a low salinity medium with a sodium concentration up to about 6.58 g/l and, preferably, up to about 4.61 g/l. These sodium concentrations of the medium correspond to the X-axis data points plotted in Figure 7 (See Figure 7; Declaration of William R. Barclay, paragraph 11; and Example 8, page 54, lines 6-12). Seawater has a sodium concentration of about 10.71 g/l (See Declaration of William R. Barclay, paragraph 12). The ability of the microorganisms of the present invention to grow at low salinities provides a strong economic advantage when considering commercial production, both because of the corrosive effects of saline waters on metal fermentors, and because of problems associated with the disposal of saline waters. (See page 54, lines 8-12).

Rejection of Claims 4-7 Under 35 U.S.C. §103

Claims 4-7 are rejected under 35 U.S.C. §103 as being unpatentable over Long taken with Chang et al. and Traitler et al. The Examiner states that the claimed invention is drawn to a food product which contains omega-3 fatty acids for microorganisms from the order Thraustochytriales and further consisting of an antioxidant. The Examiner takes the position that while Long fails to teach an antioxidant in the food products, extruding the food product, or the packaging of the food product under non-oxidizing conditions, Chang et al. do teach that the stability of omega-3 fatty acids can be increased with the addition of an antioxidant and that Traitler et al. teach protecting oils containing polyunsaturated fatty acids from oxidation by using antioxidants. The Examiner also takes the position that Traitler et al. teach a nutritive composition containing fatty acids which is extruded prior to solvent extraction and that, therefore, it would have been obvious to one of ordinary skill in the art to employ an antioxidant as taught by Chang et al. or Traitler et al. in a food product taught by Long and expect successful results in terms of improved stability.

Applicant respectfully traverses the Examiner's rejection. Applicant respectfully submits that as the Examiner concedes, Long fails to teach the use of an antioxidant, extrusion and packaging of the food product under non-oxidizing conditions. Applicant reiterates the arguments set forth above with respect to Long.

Applicant respectfully submits that Chang et al. teach that the stability of an oil which contains, among other things, omega-3 fatty acids can be increased with the addition of an antioxidant. Specifically, Chang et al. teach a process for treating an omega-3 containing oil in order to provide improved flavor and stability of the oil. Specifically, Chang et al. teach the use of Rosemary or a vegetable oil, preferably corn oil, for use as the antioxidant.

Doesn't
matter what
other benefits
are derived

no support in Specification
must not be critical if can be done either way

In contrast, the present invention teaches and claims the addition of an antioxidant to an omega-3 HUFA containing whole cell microorganism and grain paste mixture prior to extrusion of the paste. The present invention teaches that the antioxidant may be added to the fermentation medium to allow the cells to take up the antioxidants and incorporate them into the cell prior to harvesting of the cells. Alternatively, the present invention teaches that the antioxidant may be added to the omega-3 HUFA food product during post-harvest processing of the cells (See page 18, line 29 to page 19, line 31 and amended Claim 4).

Applicant respectfully traverses the argument of the Examiner that Traitler et al. teach the use of antioxidants and extrusion to protect polyunsaturated fatty acid containing oils from oxidation. Applicant respectfully submits that Traitler et al. teach extraction of fatty acids, specifically, gamma-linolenic acid, versus omega-3 highly unsaturated fatty acids, from seeds of fruits of the Ribes genus. Traitler et al. teach the use of ascorbic acid or benzoic acids as the antioxidant composition to be added to the Ribes seeds (See Column 3, lines 59-61 and Column 5, lines 10-17). Specifically, Traitler et al. teach the use of an antioxidant which is added to the seeds prior to pressing of the seeds or extrusion of the seeds in order to enhance the subsequent solvent extraction of fatty acids from the seeds (See Column 5, lines 25-29). In contrast, the present invention teaches and claims the addition of an antioxidant to an omega-3 HUFA containing whole cell microorganism and grain paste mixture prior to extrusion of the paste. Applicant respectfully submits that the use of an antioxidant with Ribes seeds does not render the use of an antioxidant with a whole cell microorganism obvious. [The present invention teaches that the antioxidant may be added to the fermentation medium to allow the cells to take up the antioxidants and incorporate them into the cell prior to harvesting of the

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cells. Alternatively, the present invention teaches that the antioxidant may be added to the omega-3 HUFA food product during post-harvest processing of the cells (See page 18, line 29 to page 19, line 31 and amended Claim 4). Applicant further respectfully submits that Traitler et al. do not teach extrusion of whole cell microorganisms. In contrast, the present invention teaches extrusion of a whole cell microorganism and a grain paste mixture (See page 21, lines 22-29 and Claim 7).

Applicant further respectfully submits that the present invention teaches and claims extrusion in order to manipulate the bioavailability of the omega-3 HUFAs contained in the whole cell microorganisms to the end user (See page 20, lines 5-29; page 21, line 22 to page 22, line 11 and amended Claim 7). In contrast, Traitler et al. teach extrusion in order to enhance the subsequent solvent extraction of fatty acids from Ribes seeds. The present invention also teaches extrusion of a whole cell microorganism and grain paste mixture in order to dry the paste and thereby inexpensively remove the water content of the paste. The present invention further teaches use of extrusion in order to enhance the stability of the whole cell and grain paste mixture because the heat used during the extrusion process destroys enzymes which cause oxidation of the paste. The extrusion process further protects the whole cells from reaction with air which causes oxidation of the omega-3 HUFAs, because the extruded matrix causes the grain paste to physically surround the whole cells which helps prevent oxygen from readily reaching the omega-3 highly unsaturated fatty acids contained within the cells (See page 21, lines 33-38). Natural antioxidants in the grain paste mixture may also contribute to the stability of the omega-3 highly unsaturated fatty acids (See page 21, lines 32-38).

The Examiner further takes the position that it also would have been obvious to one of ordinary skill in the art to apply a

known technique, such as extruding, to a food composition taught by Long and expect successful results. Furthermore, that the choice of the form of the microorganism (whole or lysed), as well as, the means of packaging is a matter of judicious selection on the part of the ordinary artisan in optimization of conditions. Applicant respectfully traverses the Examiner's rejection. Applicant respectfully submits that the present invention teaches that direct extrusion of the whole cell and grain paste mixture allows manipulation of the bioavailability of the omega-3 HUFAs for feed supplement applications by degree of cell rupture. The desired degree of cell rupture will depend on various factors, including the acceptable level of oxidation (increased cell rupture increases likelihood of oxidation) and the required degree of bioavailability by the animal consuming the extruded material (See page 22, lines 2-11).

within
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Rejection of Claims 1-3, 8 and 10 Under 35 U.S.C. §103

Claims 1-3, 8 and 10 are rejected under 35 U.S.C. §103 as being unpatentable over Ellenbogen et al. taken with Kyle. Applicant notes that as previously set forth, Claim 10 has been withdrawn from consideration. The Examiner takes the position that Ellenbogen et al. teach the production of omega-3 highly unsaturated fatty acids from the genera Thraustochytrium and Schizochytrium and that Kyle teaches the screening, selecting, and improving of the yields of eicosapentaenoic acid (EPA) in microalgal species as a possible alternative to fish oil for a source of EPA in the food industry and that, therefore, Kyle provides the motivation to screen, select and improve culturing conditions to increase the yields of EPA in the microalgal genera taught by Ellenbogen et al., as well as, its application in the food industry.

Applicant respectfully traverses the Examiner's rejection. The Examiner concedes that Ellenbogen et al. fail to teach the use of Thraustochytrium and Schizochytrium microorganisms as a source for omega-3 fatty acids for use in a food composition. Applicant respectfully submits that the present invention teaches and claims the use of whole cell microorganisms of the order Thraustochytriales as a source of omega-3 highly unsaturated fatty acids in a food product (Claims 1-8), the culturing of these microorganisms in a medium with a low salinity concentration (Claims 54, 55, 58 and 59) and that these microorganisms have an omega-3 highly unsaturated fatty acid content of greater than 6.7 percent of total cell dry weight (Claims 56 and 62). Applicant further respectfully submits that Ellenbogen et al. studied the production of omega-3 fatty acids in Thraustochytrium and Schizochytrium for the purposes of determining the phylogeny (the evolution of a genetically related group of organisms as distinguished from the development of the individual organism) of these microorganisms. Ellenbogen et al. report the fatty acid profile of these microorganisms as the relative percent of the total fatty acid content of these microorganisms. As set forth in the specification of the present invention, the omega-3 fatty acid content of a microorganism as relative percent of total fatty acids is not useful information unless data on the total lipid content is also available. Without information on the total lipid content of the microorganism, the potential of an organism for use in the production of fatty acids cannot be evaluated. As of the filing date of the above-identified application, such information was not contained in the prior art (See page 6, line 29 to page 7, line 16). The present invention teaches a collection and screening process, as set forth in Example 1 (pages 33-34) to isolate microorganisms which have an enhanced omega-3 highly unsaturated fatty acid content. The microorganisms were isolated by the method

of Example 1 and then analyzed for total lipid content and also for total omega-3 highly unsaturated fatty acid content by the method of Example 5 (pages 40-42). The results of the total lipid content of the total omega-3 highly unsaturated fatty acid content are set forth in Tables 3 and 4 and also in Figures 2-4. Therefore, Applicant respectfully submits that the teaching by Ellenbogen et al. that Thraustochytrium and Schizochytrium produce some omega-3 HUFAs does not render obvious the use of whole cell microorganisms of the order Thraustochytriales as a source of omega-3 HUFAs for use in a food composition, the culturing of these microorganisms in a medium with a low salinity concentration or that these microorganisms produce an omega-3 highly unsaturated fatty acid content greater than about 6.7 percent of total cell dry weight as taught and claimed by the present invention.

The Examiner takes the position that because Kyle teaches the screening, selecting and improving of the yield of EPA in microalgal species, that Kyle provides the motivation to do so in the microalgae taught by Ellenbogen et al., as well as, its application in the food industry. Applicant respectfully traverses the Examiner's rejection. Applicant respectfully submits that Kyle teaches away from the present invention, because Kyle teaches decreasing the temperature of the culture by 10°C in order to induce the production of omega-3 fatty acids. In contrast, the present invention does not teach decreasing the temperature of the culture, but rather teaches that decreasing the temperature of the culture in turn decreases the growth of the microorganisms and that, as a result, the overall omega-3 fatty acid yield is not significantly increased (See page 5, line 33 to page 6, line 3). Furthermore, Kyle teaches that the omega-3 fatty acid content achieved was up to one quarter (or 25%) of the total fatty acid content. In contrast, the present invention teaches that the omega-3 fatty acid content achieved by the microorganisms (claimed

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in Claims 57, 61 and 64) of the present invention is 55.8%, 49.7%, 67.3%, 50.0% and 79.5% of the total fatty acid content for the strains identified by ATCC Accession Numbers 20888, 20889, 20891 and 20892, respectively (See Table 3, pages 43-45).

Rejection of Claims 4-7 Under 35 U.S.C. §103

Claims 4-7 are rejected under 35 U.S.C. §103 as being unpatentable over Ellenbogen et al. taken with Kyle as applied to Claims 1-3, 8 and 10 above, and further in view of Chang et al. and Traitler et al. In this rejection, the Examiner relies upon the argument previously set forth with regard to Chang et al. and Traitler et al. The Examiner also takes the position that it would have been obvious to one of ordinary skill in the art to utilize antioxidants, as well as, extruding methods as taught by Chang et al. and Traitler et al. in the food composition taught by Ellenbogen et al. and Kyle with a high expectation of successful results. The Examiner further takes the position that the choice of the form of the microorganism (whole or lysed), as well, as the means of packaging is a matter of judicious selection on the part of the ordinary artisan in optimization of conditions.

Applicants respectfully submit that in order for a combination of references to render an invention obvious, it must be obvious that the teachings can be combined. In re Avery, 518 F.2d 1228, 186 U.S.P.Q. 161 (C.C.P.A. 1975). The mere fact that references can be combined does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. Berghauser v. Dann, Commissioner of Patents, 204 U.S.P.Q. 393 (D.C.D.C. 1979). Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching suggestion or incentive supporting the combination. In re Geiger, 2 U.S.P.Q.2d 1276 (C.A.F.C. 1987). The references, viewed by themselves and not in retrospect, must

suggest doing what applicant has done. In re Shaffer, 229 F.2d 476, 108 U.S.P.Q. 326 (C.C.P.A. 1956); In re Skoll, 523 F.2d 1392, 187 U.S.P.Q. 481 (C.C.P.A. 1975). In deciding obviousness one must look at prior art from the vantage point in time prior to when an invention was made; hindsight obviousness after the invention has been made is not the test. In re Carroll, 601 F.2d 1184, 202 U.S.P.Q. 571 (C.C.P.A. 1979). The mere fact that it is possible for two isolated disclosures to be combined does not render the result of that combination obvious absent a logical reason of record which justifies the combination. In re Regel et al., 526 F.2d 1399, 188 U.S.P.Q. 136 (C.C.P.A. 1975).

Applicant respectfully traverses the Examiner's rejection. Applicant reiterates all arguments set forth above with respect to Chang et al., Traitler et al., Ellenbogen et al. and Kyle. Applicant respectfully submits that Ellenbogen et al. do not teach omega-3 fatty acid containing microorganisms or omega-3 fatty acid oils for use in food products. Applicant further respectfully submits that Kyle teaches the use of omega-3 fatty acid oils extracted from microorganisms in order to replace omega-3 fatty acid containing fish oils. Applicant also respectfully submits that Kyle does not teach the use of the microorganisms themselves in order to achieve an omega-3 fatty acid containing food product.

Applicant respectfully traverses the position of the Examiner that the choice of the form of the microorganism (whole or lysed), as well as, the means of packaging is a matter of judicious selection on the part of the ordinary artisan in optimization of conditions. Applicant respectfully submits that if the choice of the form of the microorganism was merely a matter of judicious selection, then the use of whole or lysed microorganisms would have been taught by the prior art. However, each of the prior art references cited by the Examiner teaches away from the present invention. Specifically, each of the prior art references cited by

the Examiner teaches extraction of an omega-3 fatty acid oil from a microorganism or other source. In contrast, the present invention teaches that, preferably, the whole cell microorganism containing the omega-3 fatty acids, versus the omega-3 fatty acid extracted from the microorganism, is directly incorporated into a food product. Furthermore, Claims 1-8 and 53-57 claim whole cell microorganisms as a food product containing a source of omega-3 highly unsaturated fatty acids. As previously discussed, the use of the whole cell microorganism has many attendant advantages, such as prevention of oxidation. Also, because the whole cell microorganism already has a high omega-3 HUFA content, the omega-3 HUFAs do not need to be extracted, but rather the whole cell microorganism can be directly incorporated into feed material to produce an omega-3 enriched feed.

In view of the foregoing amendments and remarks, Applicant submits that all pending claims are in condition for allowance. Consideration of the above and withdrawal of all rejections are hereby requested.

Applicant wishes to thank the Examiner for extending the courtesy of a telephone conference on April 30, 1991, during which Applicant's Attorney, Brenda Speer, and the Examiner discussed the possibility of scheduling a personal interview for May 29, 1991. However, at that time the Examiner indicated that in order to expedite prosecution, an interview would be more appropriate after the issuance of a second Office Action. If the Examiner decides to issue the second Office Action as a final Office Action, then Applicant requests that the Examiner notify the Applicant by telephone of her intentions prior to any such issuance in order that Applicant may have the opportunity to discuss with the Examiner any such final Office Action.

Applicant submits herewith a Petition for Extension of Time for one month from May 14, 1991, to June 14, 1991, to respond to

the Office Action dated February 14, 1991. Applicant submits herewith a check in the amount of \$50.00 to cover the extension fee. Please credit any overpayment or debit any underpayment to Deposit Account No. 19-1970.

Applicant believes no fees are due pursuant to 37 C.F.R. §1.16 for newly added Claims 53-64, because Applicant has withdrawn from consideration a greater number of claims. However, if Applicant's belief is incorrect, then please debit any under payment to Deposit Account No. 19-1970.

A Declaration of William R. Barclay and a Supplemental Information Disclosure Statement also accompanying this Amendment and Response.

Respectfully submitted,

SHERIDAN, ROSS & MCINTOSH

Date: 5/20/91

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